Temporal and spatial factors contributing to the variability of crAssphage concentrations measured at wastewater treatment plants

University of Cincinnati Chemical and Environmental Engineering Graduate Seminar

Corinne Wiesner-Friedman, Ph.D.

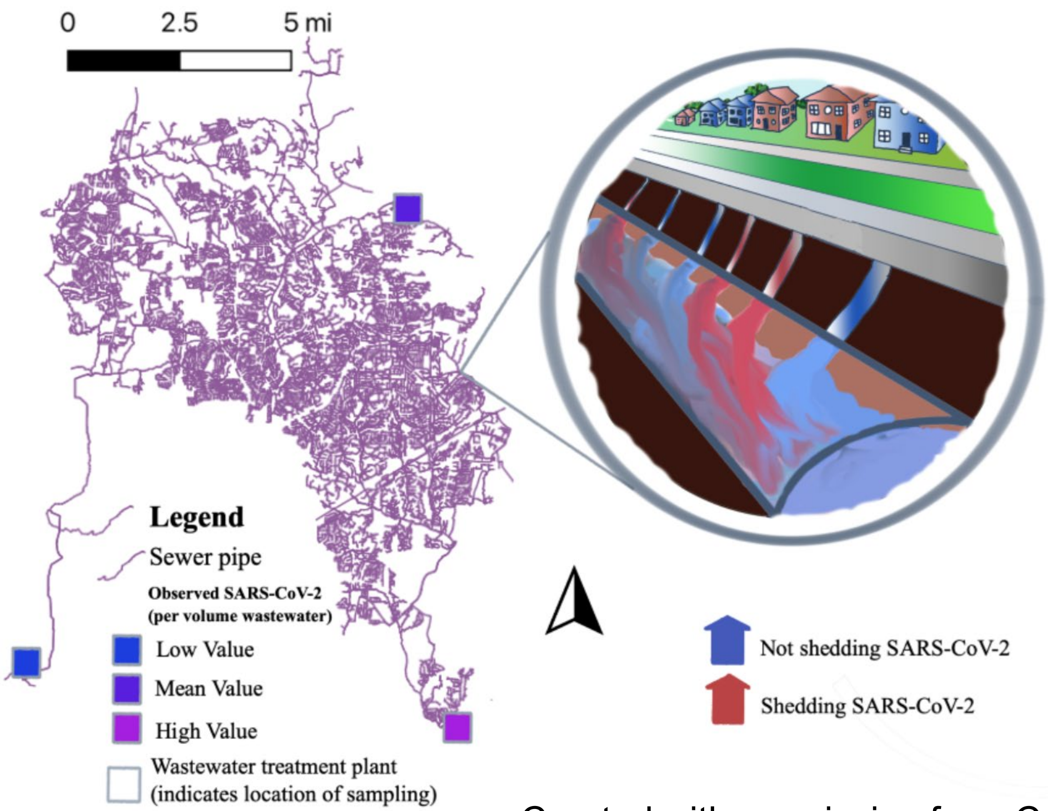
ORISE Postdoctoral Researcher

Office of Research and Development
Center for Environmental Solutions and Emergency Response

Wastewater - based epidemiology and surveillance

What can wastewater say about population health?

Wastewater -based surveillance



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Factors affecting the fate and transport of microbes/microbial responses

<u>Contribution Processes</u>

- Loadings at nodes connected to sewers (i.e., location and time)
- Decay
- Dilution
- Absorption, adsorption into sediments
- Persistence in biofilms
- Residence time
- **Changing case rates!!**

<u>Amplification Processes</u>

- Selection by other chemicals and nutrients
- Selection by other microbes/microbial responses that affect microbial community dynamics
- Climatic factors (e.g., temperature)
- Growth
- Residence time
- Physico-chemical (e.g., pH, ww temperature)

<u>Attenuation Processes</u>

- Dilution
- Decay
- Climatic factors
- Land cover (for combined systems)
- Inactivation
- Residence time
- Physico-chemical (e.g., pH, ww temperature)

(1) Balleste 2019 , (2) Ahmed 2021, (3) Pruden 2012, (4) Bertels 2022

Measurement

1. What can we measure in the wastewater to help us understand these processes that can help adjust our quantitation?
2. How can we leverage our data to best quantify our targets?

Fecal indicators and host-associated markers (e.g., crAssphage)

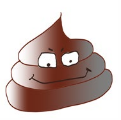
Other wastewater measurements or proxies!

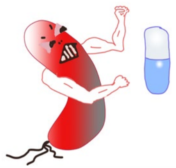
Modeling

1. What modeling approaches are currently available that characterize these processes?
2. Given that this is a relatively new field, what work needs to be done?

Current models of fate and transport of microbes/microbial responses

- Examples:[1]:
  - SWAT
  - SPARROW
  - AQUATOX
  - CE-QUA-W2
  - WASP
  - SWMM (urban)





- Developed primarily for nutrient responses, fecal indicator bacteria
- No developed for viruses/pathogens
- Resolution at the sub-watershed level
- Require prior knowledge of delivery parameters and/or concentrations of contaminants at sources

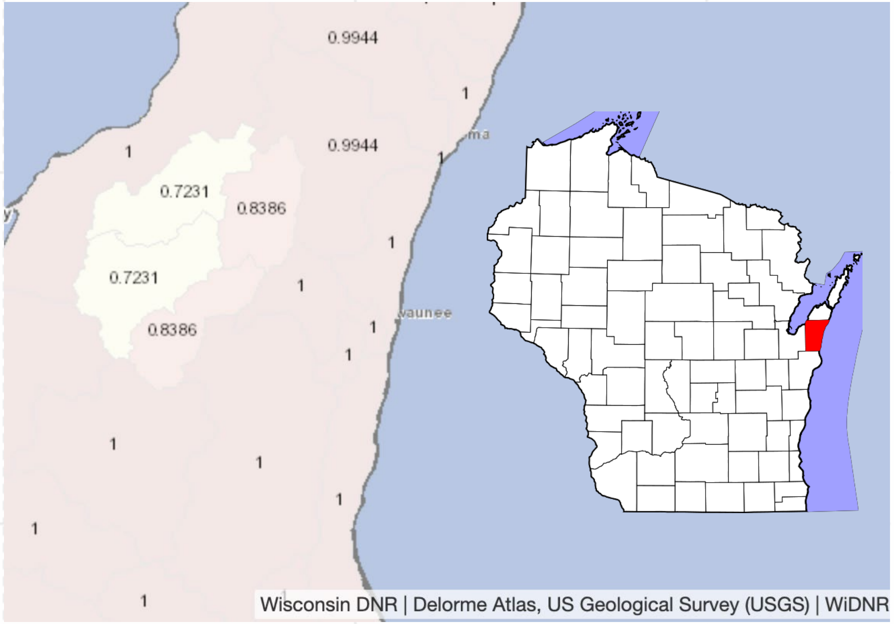


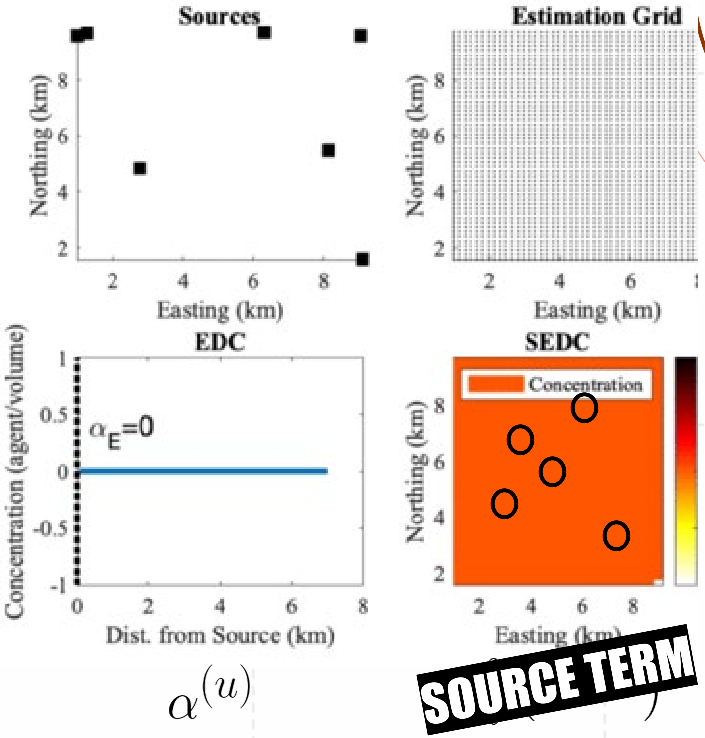
Figure 3. Catchments from the SPAtially Referenced Regression on Watershed Attributes (SPARROW) for Kewaunee County, Wisconsin

(1) Costa 2021

Flexible spatial predictor models for learning microbial spatial patterns
(Land-use regression approach)

Sum of exponentially decaying contributions (the distance away from sources expected to observe x% reduction from sources)

$\alpha^{(u)}$

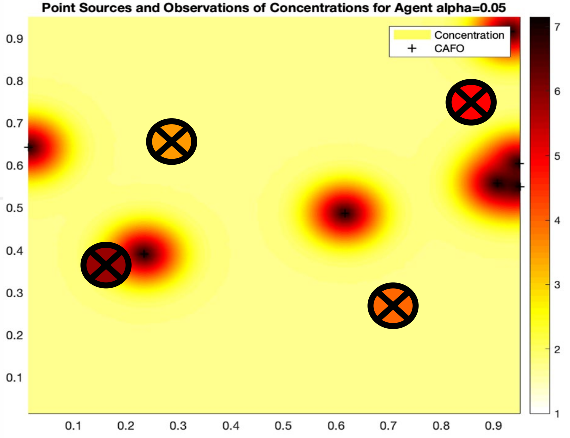


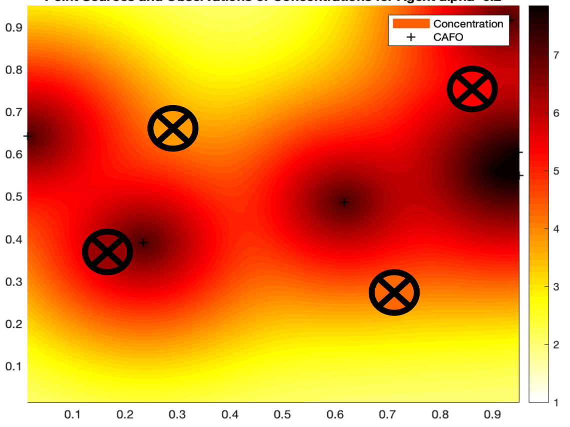
$\alpha^{(u)}$

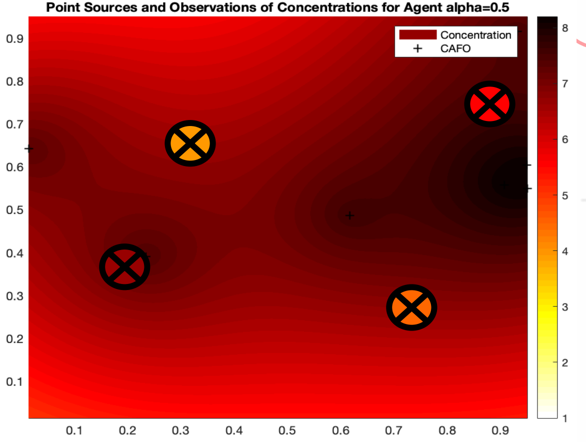
(1) Messier 2012

Selecting Hyperparameter Values for Spatial Predictor Models

Observation Locations and Response





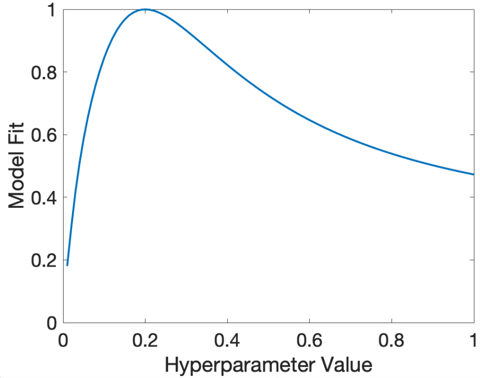


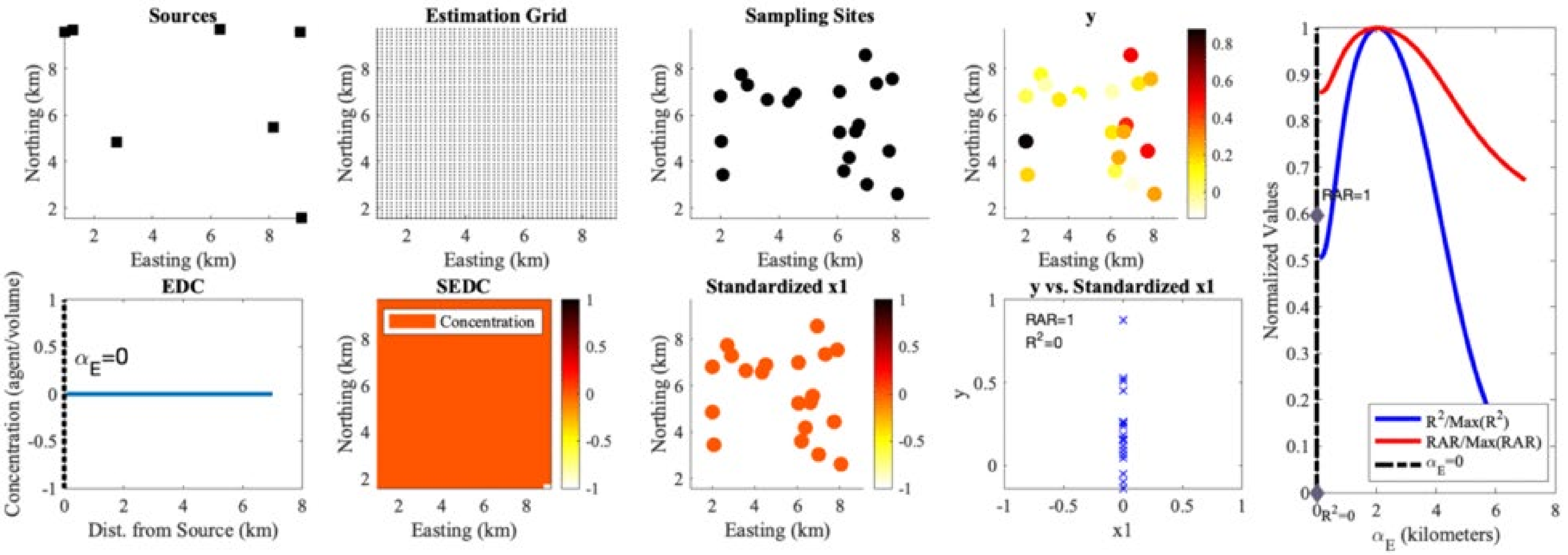
Previous hyperparameter selection

Toggle hyperparameter values

$$y_i = \beta_0 + \beta_1$$

SOURCE TERM





Sources
Estimation Grid
Sampling Sites
y
EDC
SEDC
Standardized x1
y vs. Standardized x1
Northing (km)
Easting (km)
Concentration (agent/volume)
Dist. from Source (km)
α_E=0
Concentration
RAR=1
R^2=0
x1
Normalized Values
R^2/Max(R^2)
RAR/Max(RAR)
α_E (kilometers)

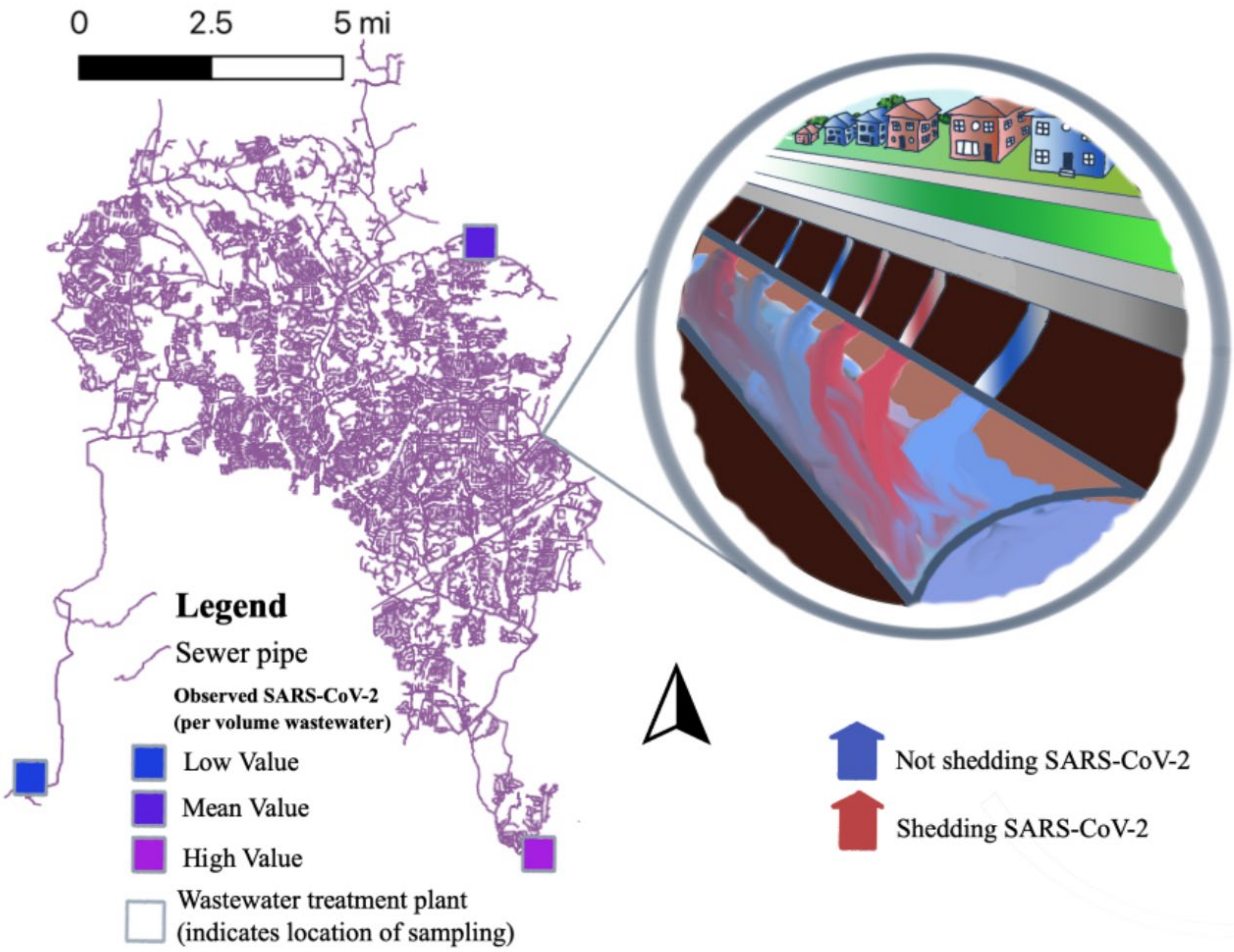
Modeled Infected Users Shedding Cor

The number of users connected to sewer segment, j

$$x_i(\alpha_{90}) = \frac{1}{q_i}\sum_{j=0}^{N} m_{0j} n_j exp(\frac{-2.3T_{ij}}{\alpha_{90}})$$

The number of target microbial responses that each customer is shedding on average in the sewershed on day *i*

$$m_{0i}n_j$$



0
2.5
5 mi
Legend
Sewer pipe
Observed SARS-CoV-2
(per volume wastewater)
Low Value
Mean Value
High Value
Wastewater treatment plant
(indicates location of sampling)
Not shedding SARS-CoV-2
Shedding SARS-CoV-2

Modeled Users Shedding Contributions

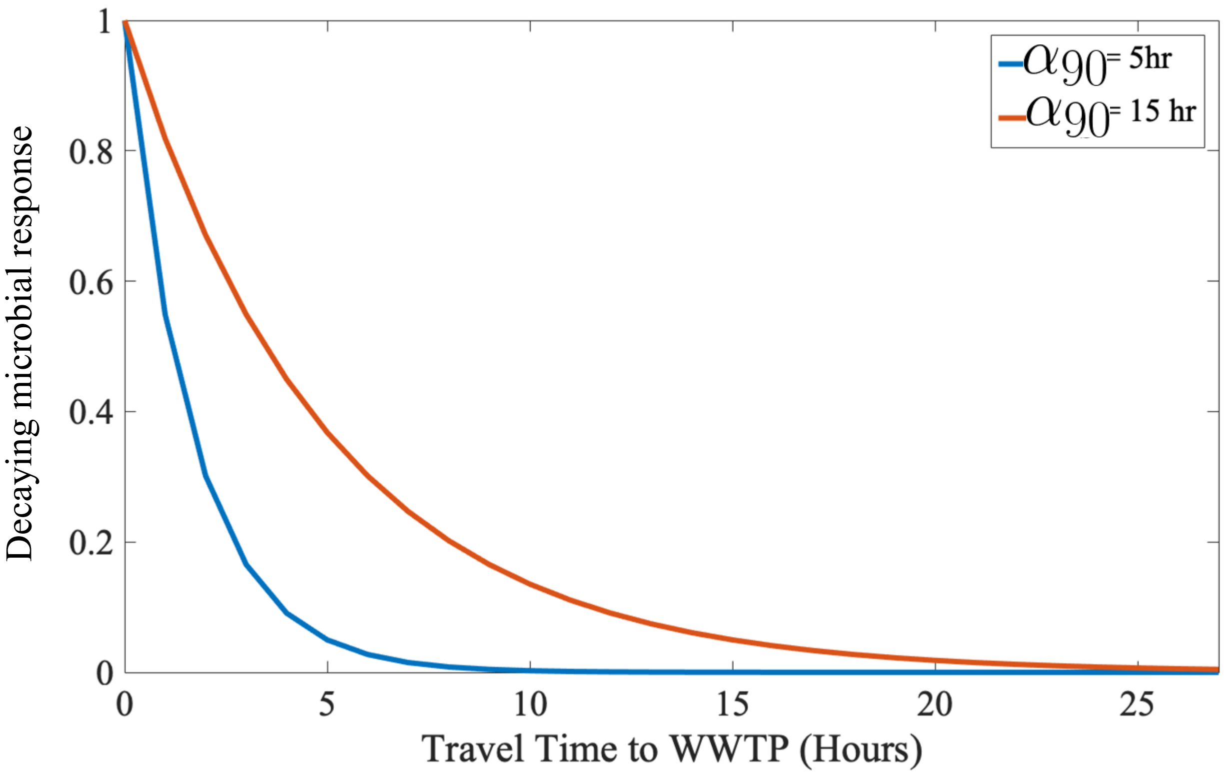
Travel time from each location where the target microbial response is being shed into the network to the sampling point

$$x_i(\alpha_{90}) = \frac{1}{q_i} \sum_{j=0}^{N} m_{0j} n_j exp(\frac{-2.3T_{ij}}{\alpha_{90}})$$

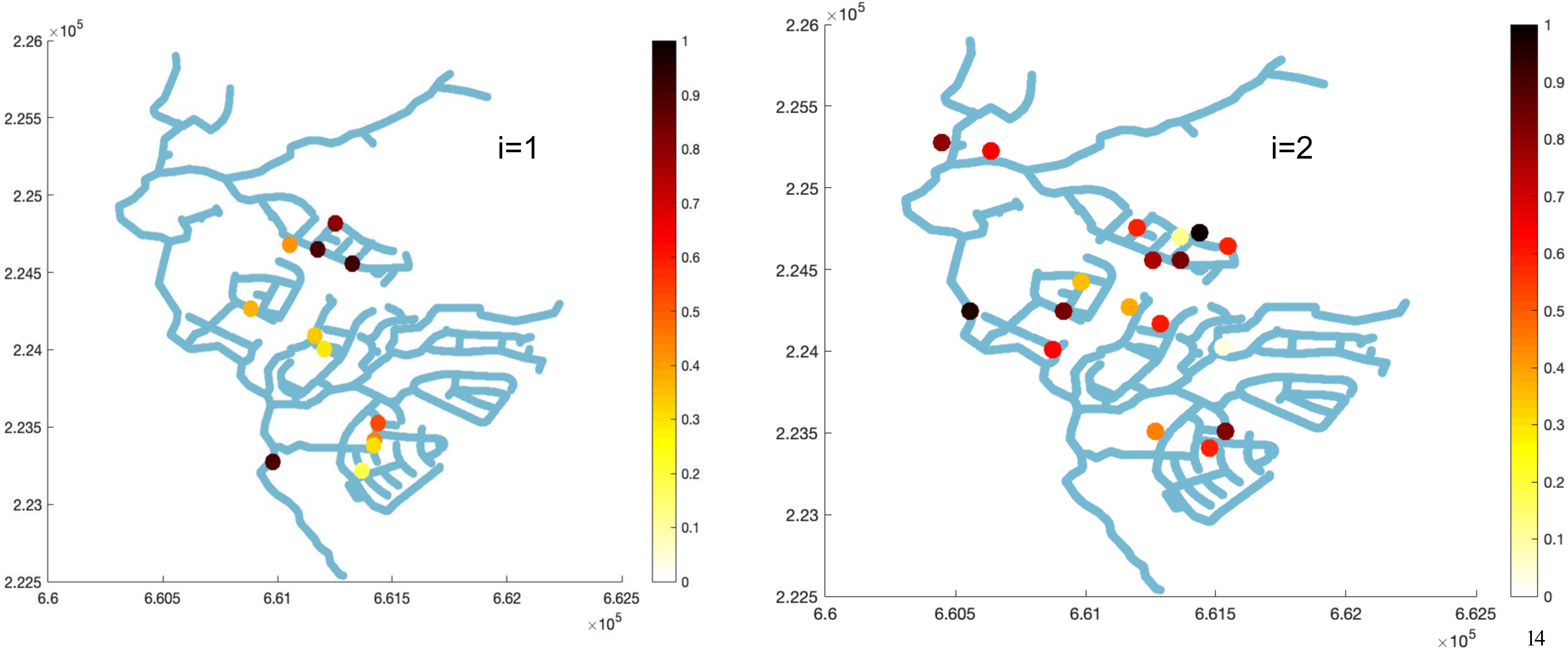
Flow at the sampling point

The travel time at which you would expect to see a 95% reduction of the target microbial response from the input point

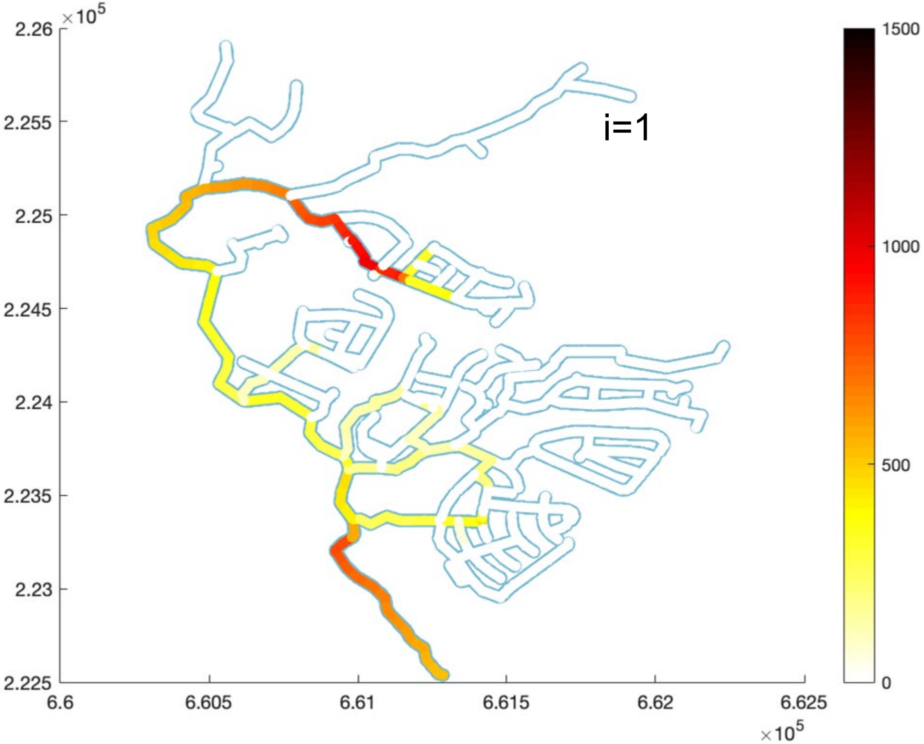
How does $\propto_{90}$ affect User Shedding

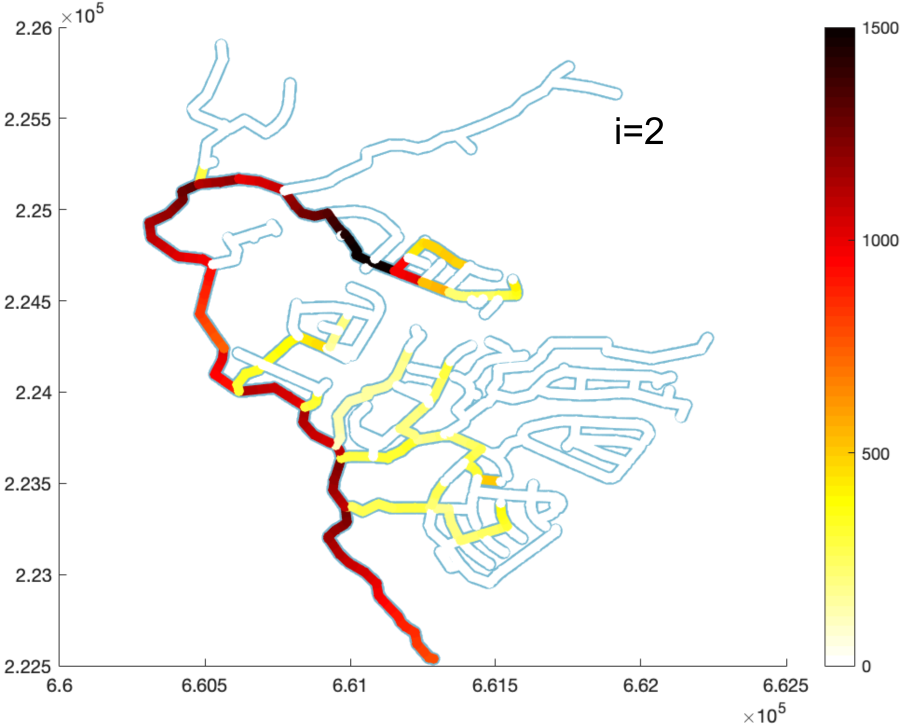


If yellow-red dots on this network equal $m_{0j} n_j$

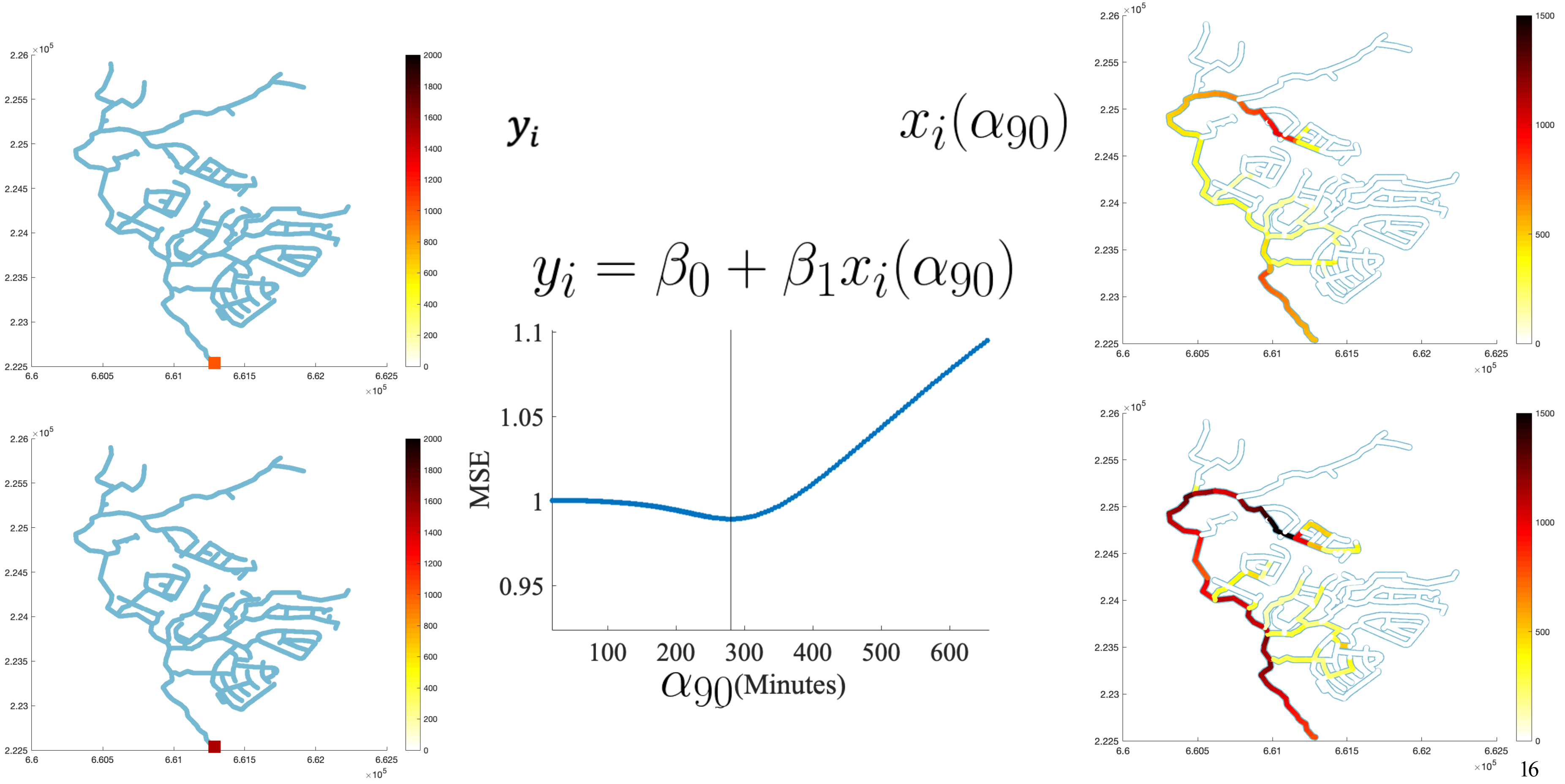


We might see time-travel decay and dilution in the sewer network that resembles this for our i=1 and i=2





Compare the observed versus predicted to find the best decay parameter



$$T_{ij} \approx D_{ij}?$$

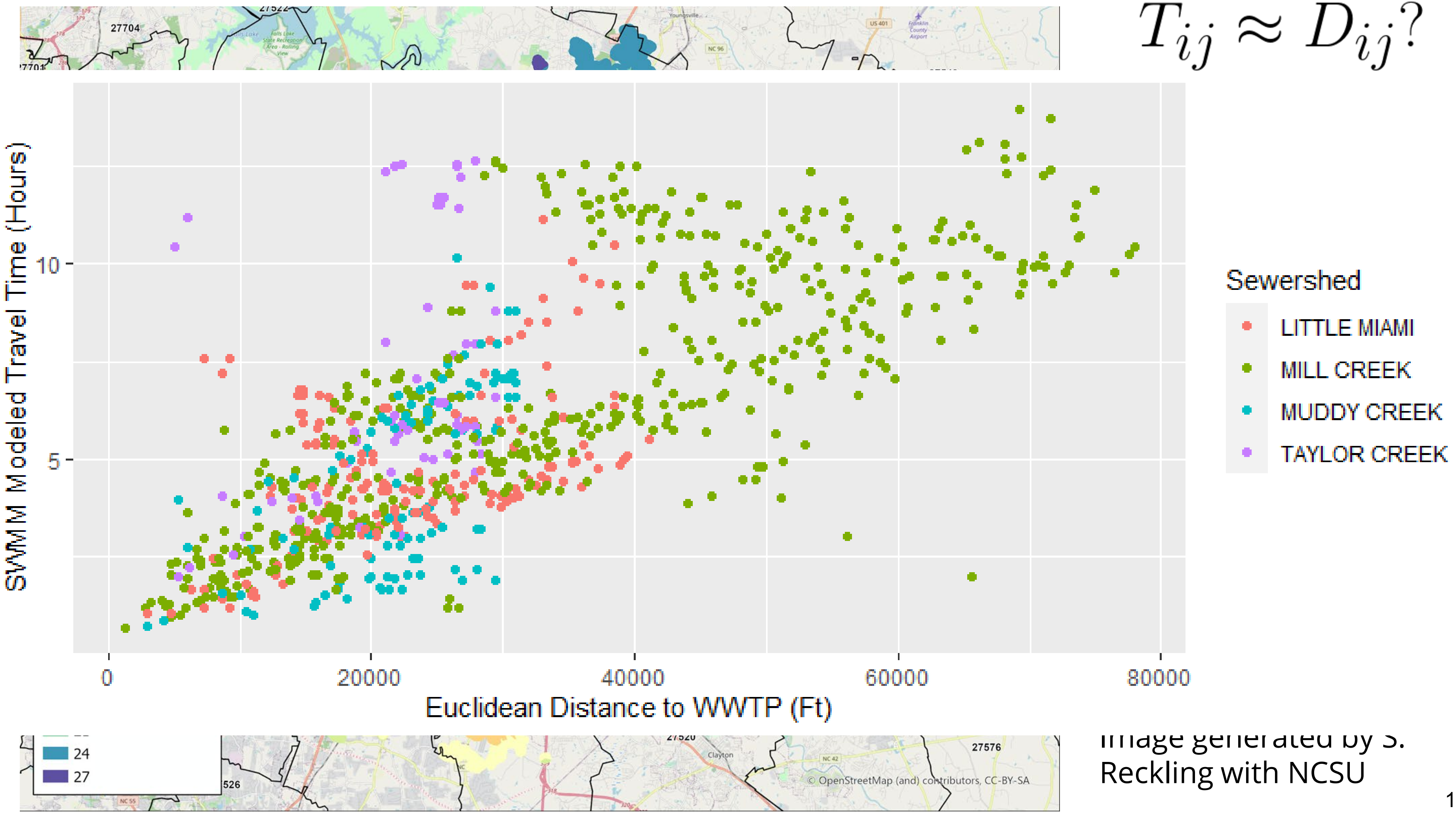


Image generated by S. Reckling with NCSU

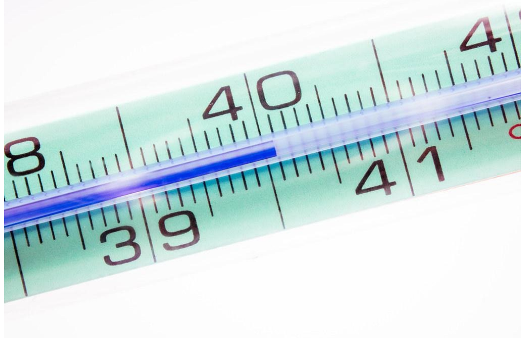
Developing a new spatial predictor models tailored to sewer networks for WBS

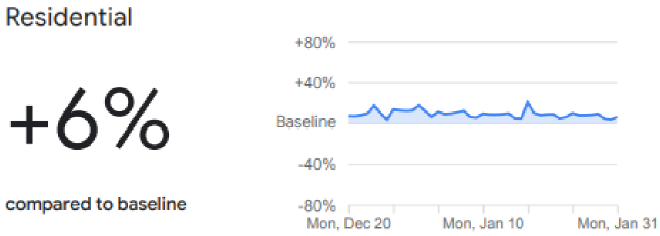
Residential Contributions to crAssphage

F $$x_i(\alpha_{90}) = \frac{1}{q_i}\sum_{j=1}^{N} m_{0j} n_j exp(\frac{-2.3T_{ij}}{\alpha_{90}})$$

F-T $$x_i(\alpha_{90}, \Delta) = \frac{1}{q_i}\sum_{j=1}^{N} m_{0j} n_j exp\left[\frac{-2.3T_{ij}}{\alpha_{90}(0) exp(\Delta Temp_i)}\right]$$

F-TM $$x_i(\alpha_{90}, \Delta, M) = \frac{1}{q_i}\sum_{j=1}^{N} m_{0j} n_j exp\left[\frac{-2.3T_{ij}}{\alpha_{90}(0) exp(\Delta Temp_i + Mres_i)}\right]$$



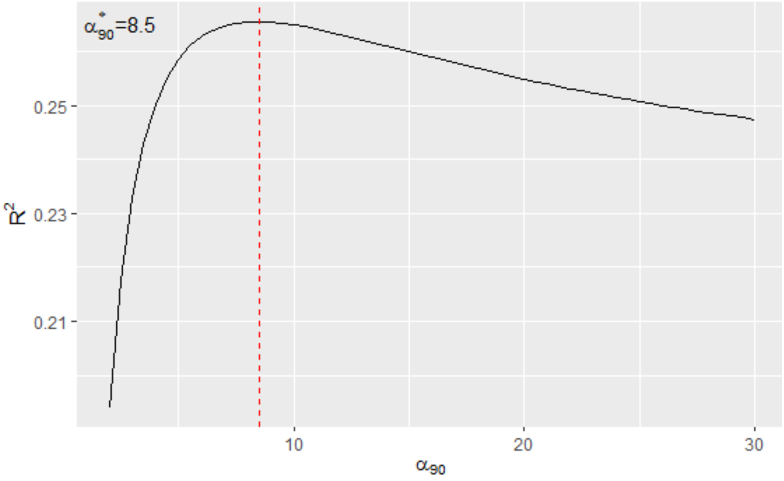


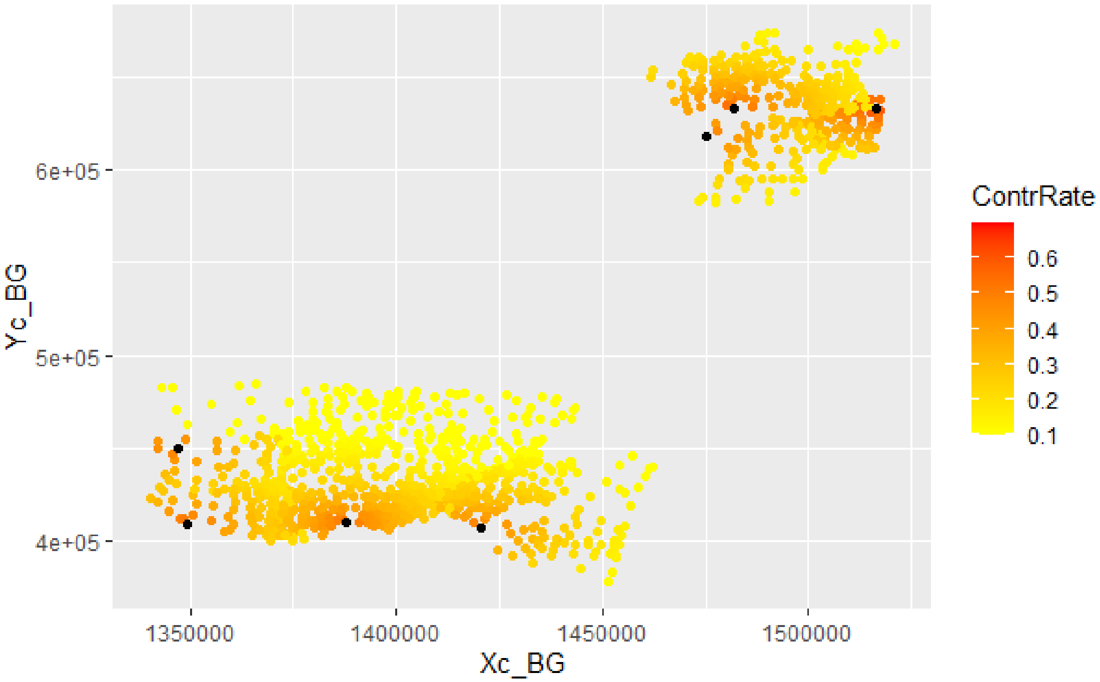
Mobility trends for places of residence.

https://www.google.com/covid19/mobility/ Accessed: 02/03/2022.

F

Model accounting for flow

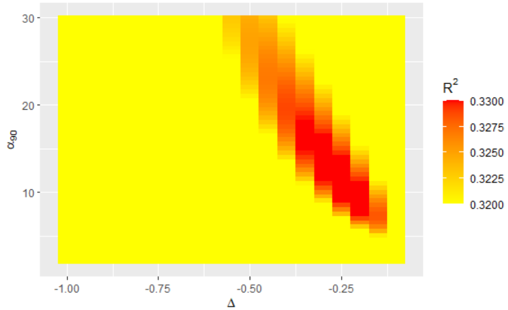




▲ modeled contributions log-transformed for normality on residuals

F-T

Model accounting for flow, and travel time influence range modified by ambient temperature

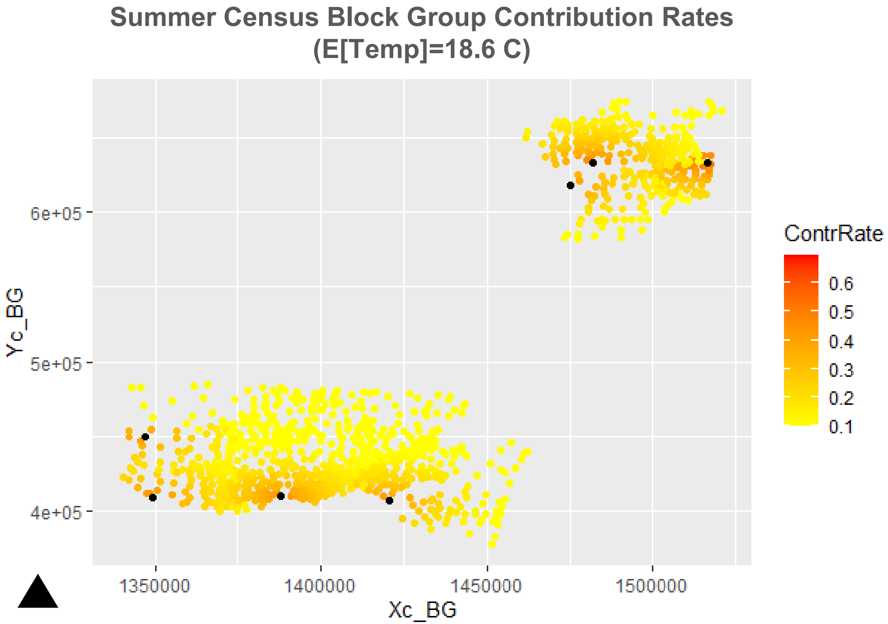


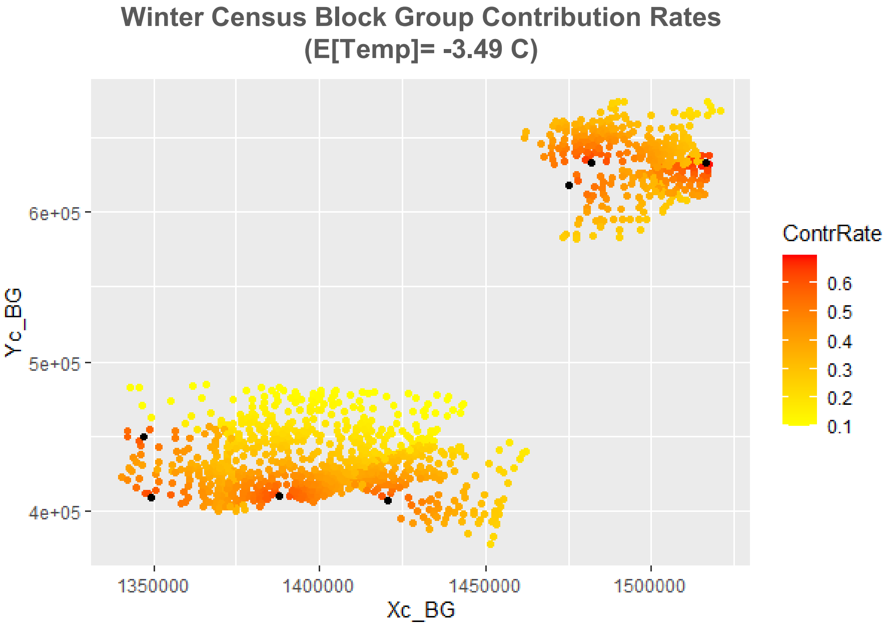
$$\alpha^*_{90} = 10.7, \Delta^* = -0.242$$

1 standard-deviation of temperature is 9.72 degrees Celsius

Minimum recorded ambient temperature = -16.0 C

Maximum recorded ambient temperature = 25.4 C

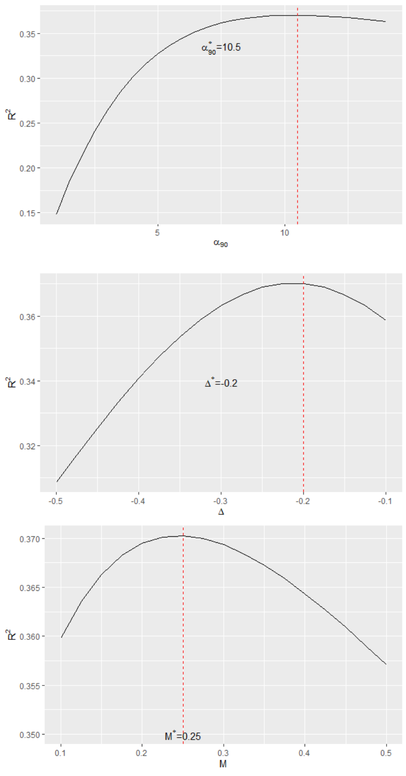


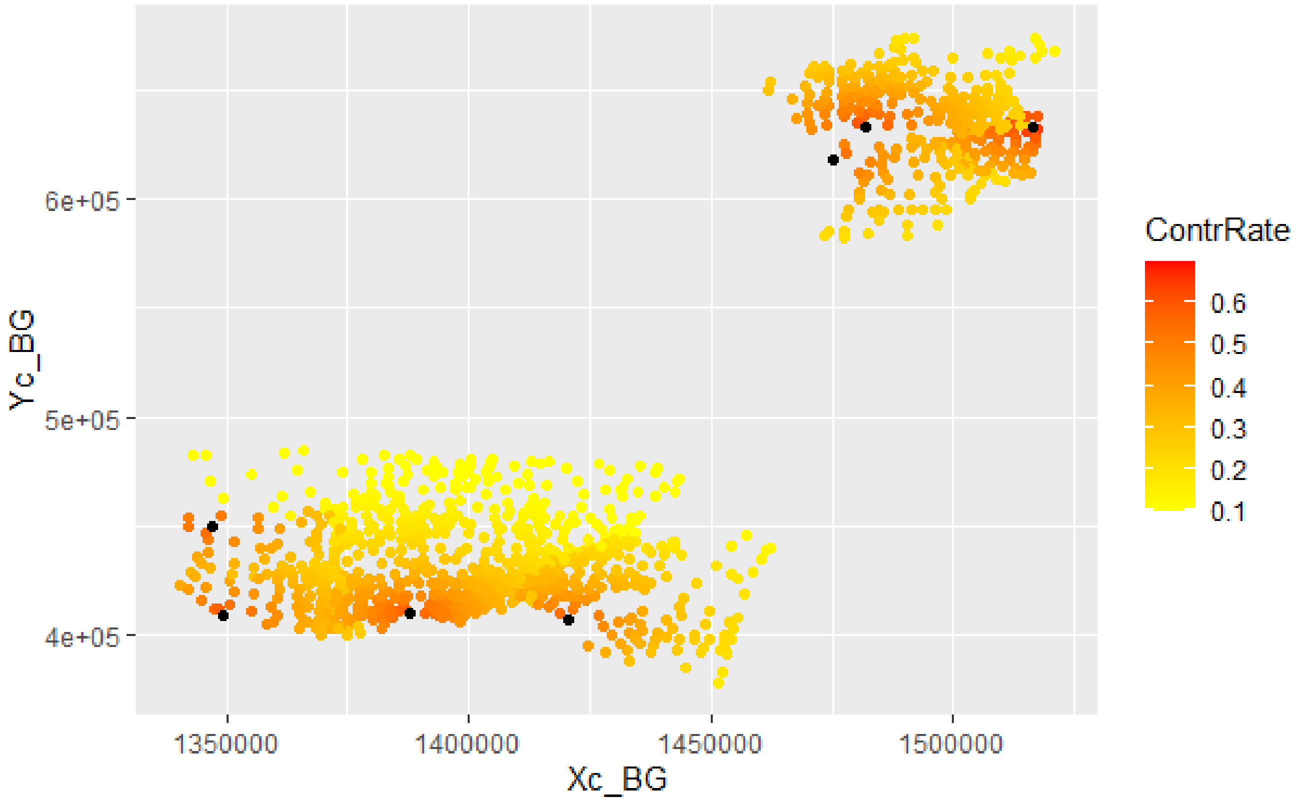


F-TM

Model accounting for flow, and travel time influence range modified by temperature and increase of those staying at home from baseline (mobility)

$$\alpha^*_{90} = 10.3, \Delta^* = -0.212, M^* = 0.245$$





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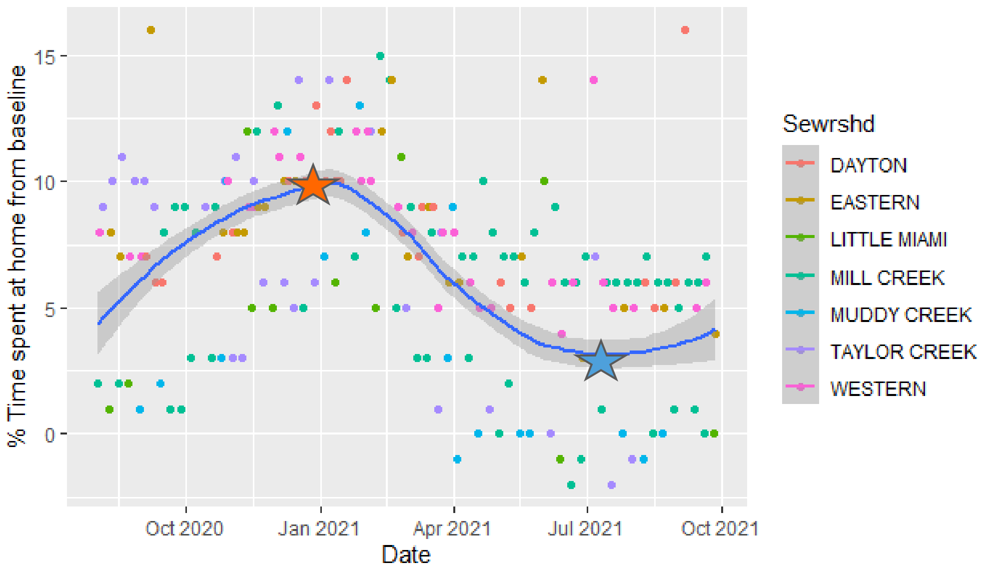
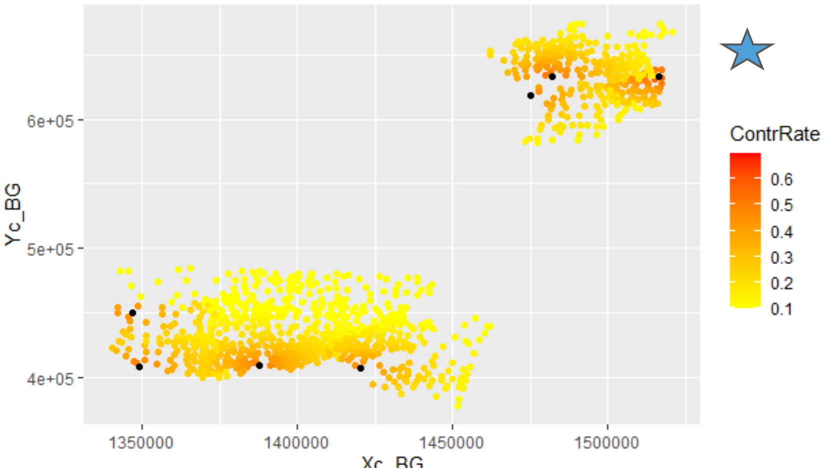
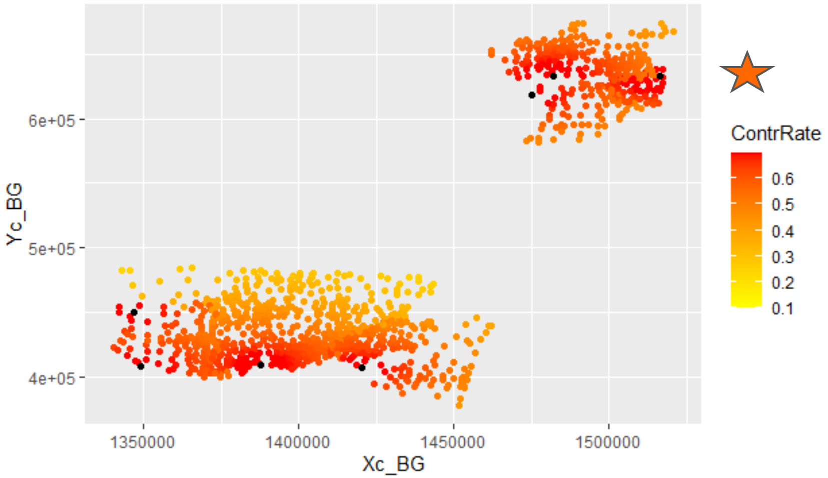
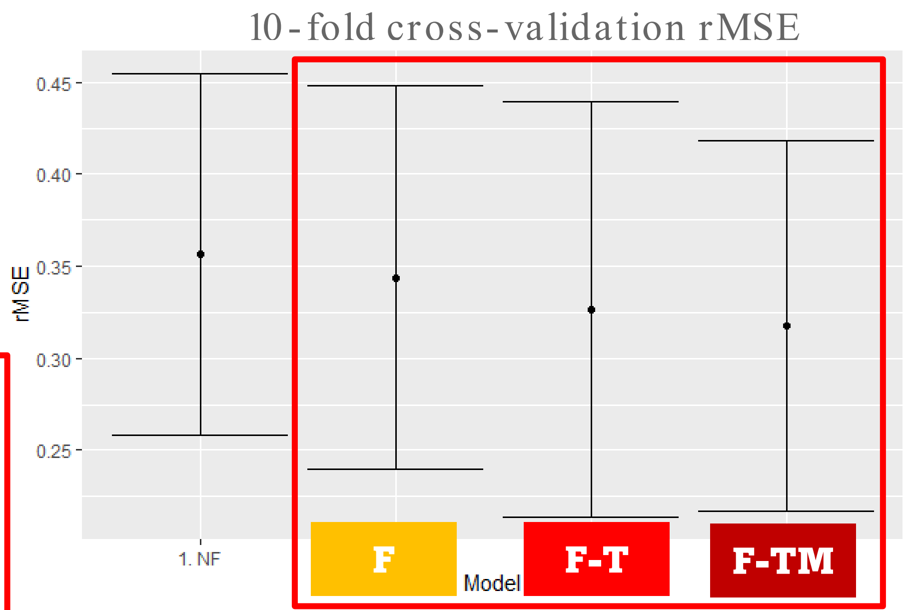






Residential Contributions Model comparison

Model	Univariate Regression Coefficient (standardized)	Univariate R-squared
1. No Flow (NF)	-0.185	0.212
2. Flow (F)	0.207	0.266
3. Flow modified by temperature (F-T)	0.231	0.333
4. Flow modified by temperature and mobility (F-TM)	0.244	0.370



Other findings/next steps

1. Commercial parcel contributions
2. Linear model with residential/commercial contributions, combined sewer, industrial flow, and pH

Developing a new spatial predictor models tailored to sewer networks for WBS

Commercial Contributions to crAssphage

F

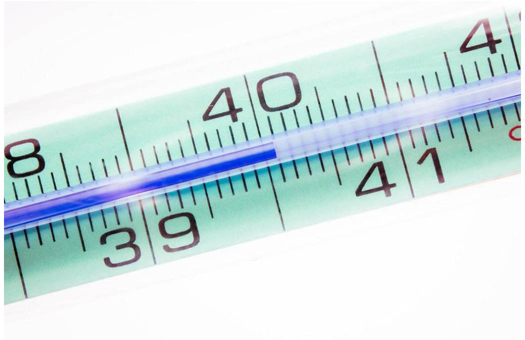
$$x_i(\alpha_{90}) = \frac{1}{q_i}\sum_{j=1}^{N} n_j exp(\frac{-2.3Tij}{\alpha_{90}})$$

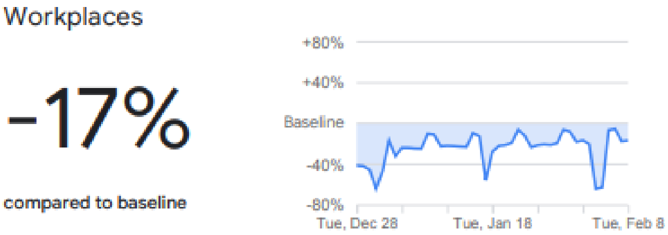
F-T

$$x_i(\alpha_{90}, \Delta) = \frac{1}{q_i}\sum_{j=1}^{N} n_j exp\left[\frac{-2.3Tij}{\alpha_{90}(0)exp(\Delta Temp_i)}\right]$$

F-TM

$$x_i(\alpha_{90}, \Delta, M) = \frac{1}{q_i}\sum_{j=1}^{N} n_j exp\left[\frac{-2.3Tij}{\alpha_{90}(0)exp(\Delta Temp_i + Mwork_i)}\right]$$



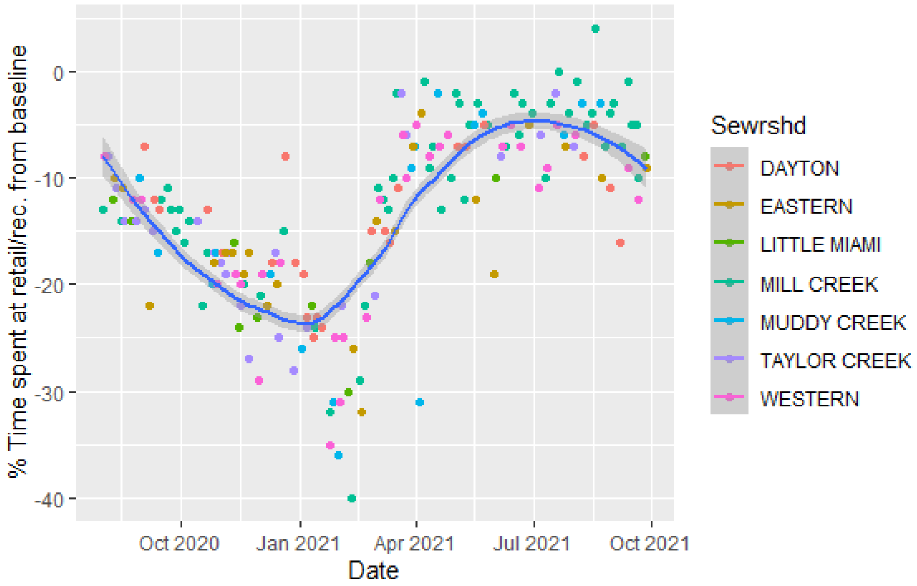


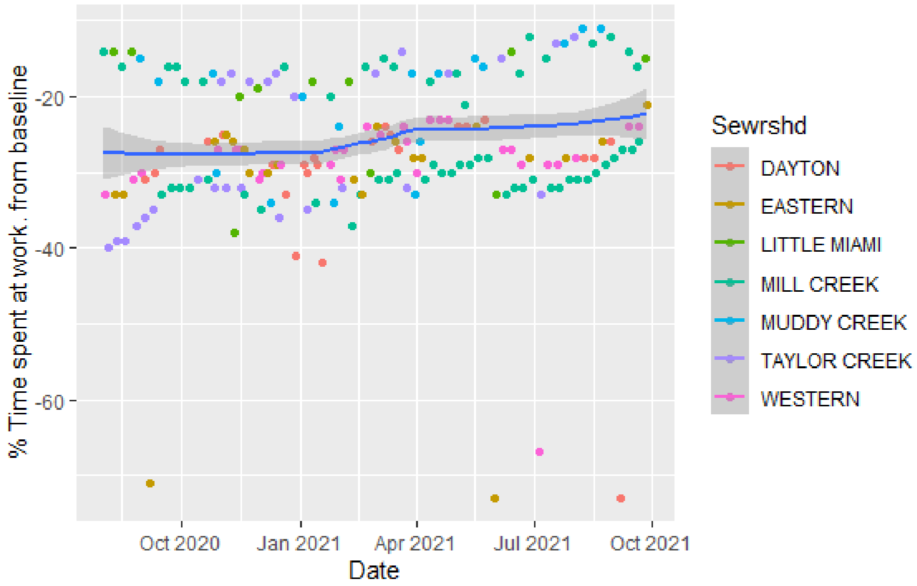
Mobility trends for places of work.

Google LLC *"Google COVID-19 Community Mobility Reports"*.
https://www.google.com/covid19/mobility/ Accessed: 02/11/2022.

Developing a new spatial predictor models tailored to sewer networks for WBS

<u>Commercial</u> Contributions to crAssphage: Mobility factors

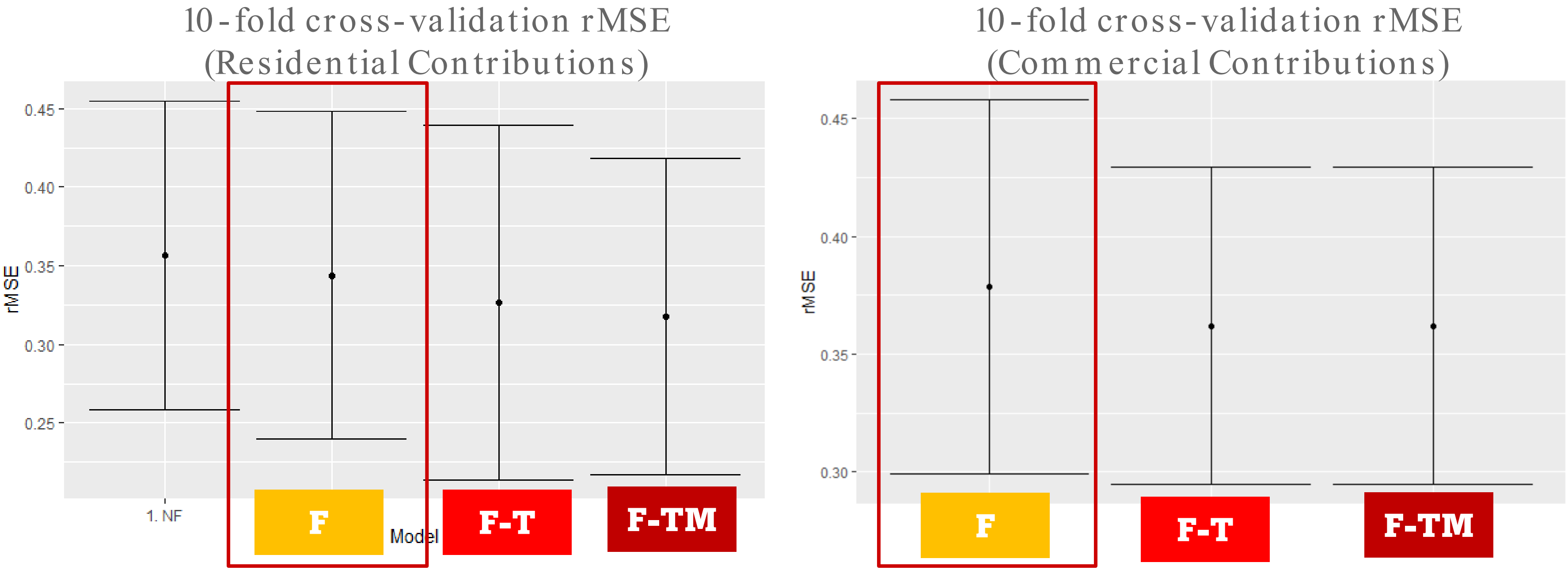




Comparing Residential and Commercial Hyperparameters and Univariate Regression

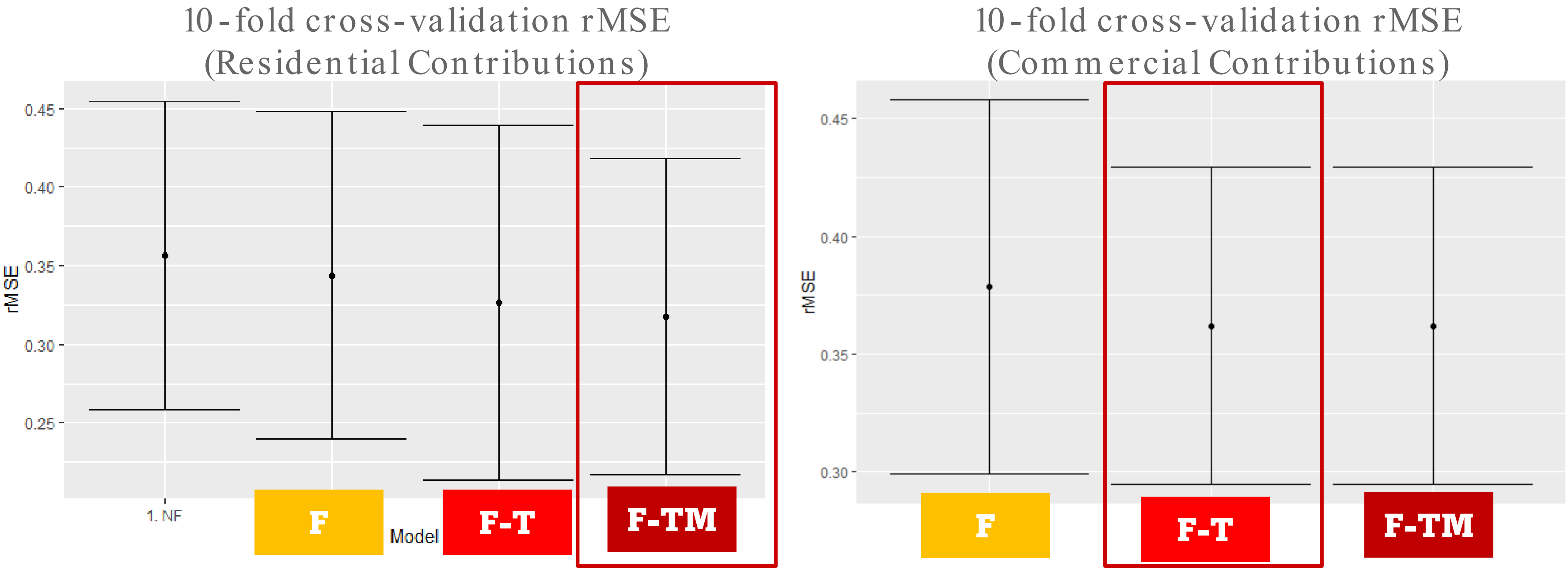
Model	Contribution Type	Hyperparameters			Univariate Regression Coefficient (Standardized)	Univariate R-squared
		α_{90} or $\alpha_{90}(0)$	Δ	M		
F	Residential (Census)	8.39	NA	NA	0.207	0.266
	Commercial (Parcels)	6.00	NA	NA	0.136	0.115
F-T	Residential (Census)	10.7	-0.242	NA	0.231	0.333
	Commercial (Parcels)	9.09	-0.451	NA	0.175	0.191
F-TM	Residential (Census)	10.3	-0.212	0.245	0.244	0.370
	Commercial (Parcels)	9.09	-0.451	0	0.175	0.191

Selecting models to use for residential versus commercial contributions



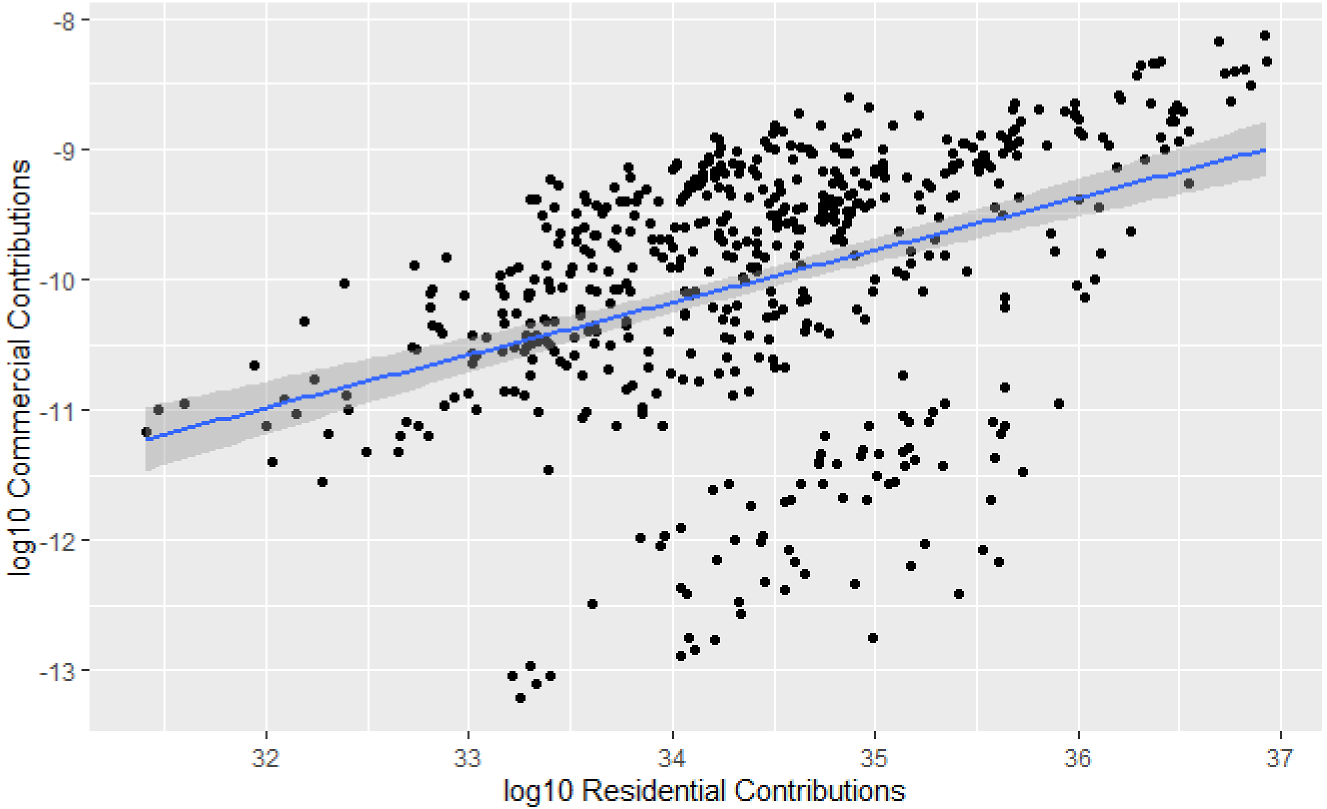
Simplest

Selecting models to use for residential versus commercial contributions



"Best"

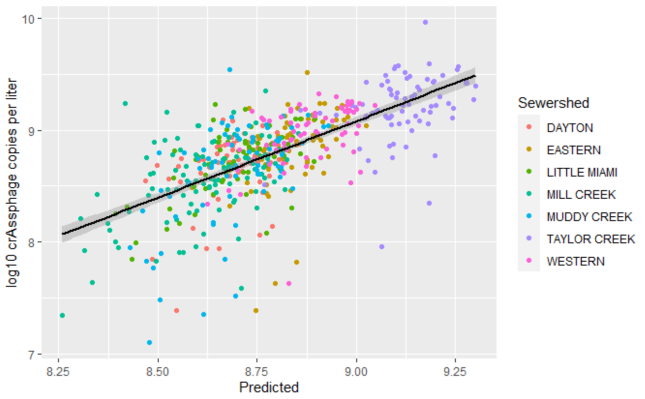
Exploring collinearity between residential and commercial contributions



ρ=0.403 (p-value<2.2e-16)

Need a regression approach to handle collinearity

Simplest Bootstrapped LASSO Regression for other factors



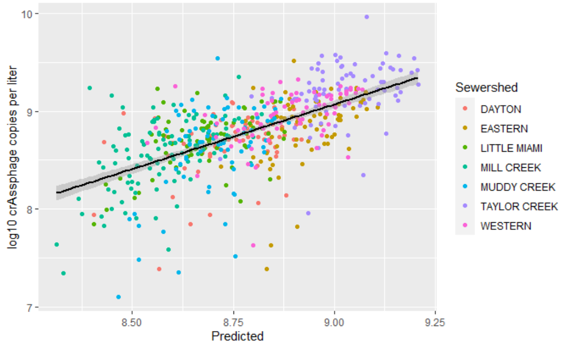
Not selected:

- pH
- Time spent retail/rec.
- Time spent transit
- Industrial flow

Variable	Log 10 res. cont. (F)	Log 10 com. cont. (F)	Temp.	Combined sewer	Log 10 48h prec.	Time spent groc./pharm.	Time spent at home
Std. Reg. Coeff. (95% BS CI)	**0.146 (0.119, 0.173)**	**0.0580 (0.02, 0.0941)**	**-0.0420 (-0.0749, -0.00903)**	-0.0545 (-0.115, 0.00637)	-0.00555 (-0.0209, 0.00979)	-0.0188 (-0.0480, 0.0105)	0.0231 (-0.00530, 0.0515)
Variable Std. Dev.	**0.258**	**0.424**	**9.72**	NA	0.211	6.18	4.29

Bootstrapped (BS) R-squared=0.404

"Best" Bootstrapped LASSO Regression for other factors



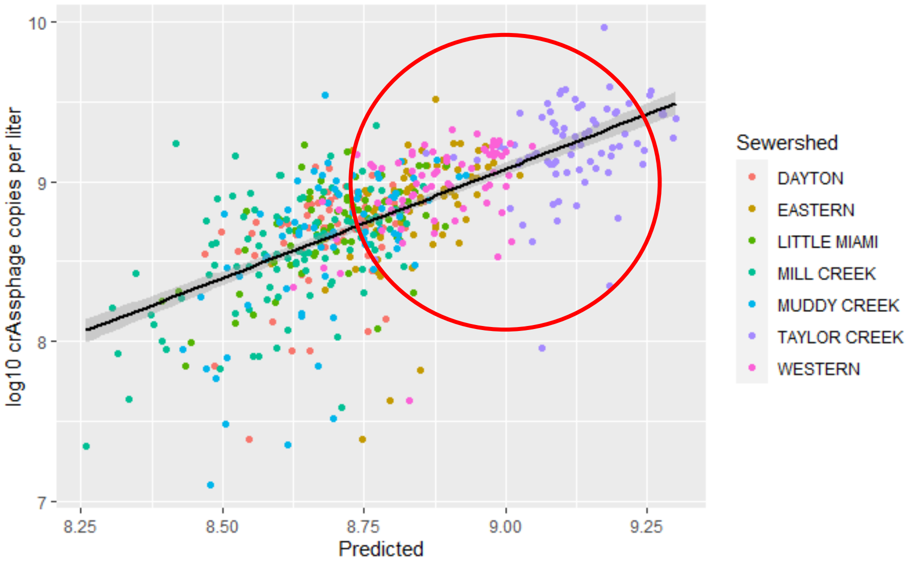
Not selected:

- pH
- Time spent transit
- Temperature

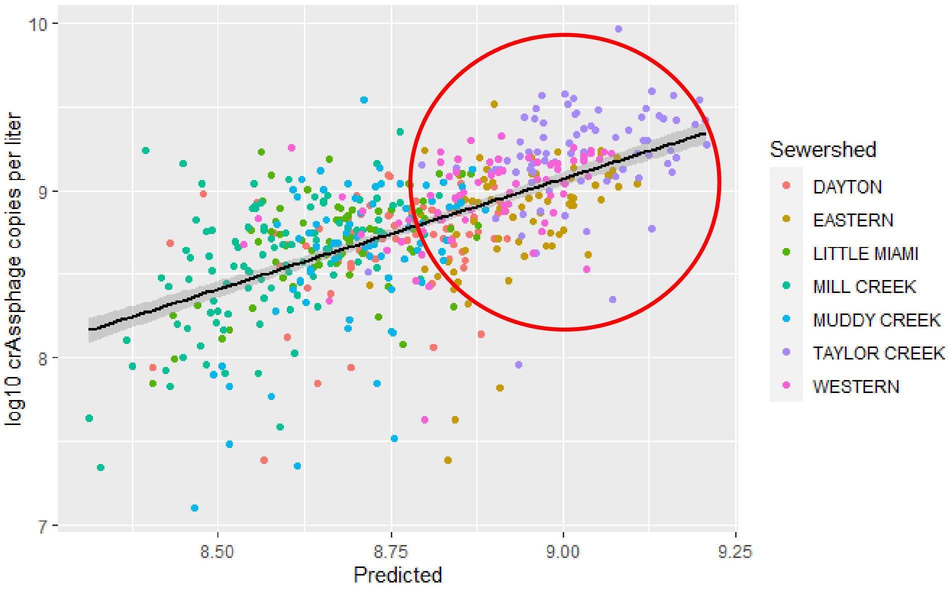
Variable	Log 10 res. cont. (F-TM)	Log 10 com. cont. (F-T)	Combined sewer	Log 48h prec.	Time spent groc./pharm.	Time spent at work	Time spent at home	Industrial Flow
Std. Reg. Coeff. (95% CI)	**0.129 (0.0723, 0.186)**	0.0365 (-0.0139, 0.0869)	**-0.142 (-0.210,-0.0738)**	**-0.0300 (-0.0573, -0.00258)**	-0.0110 (-0.0342,0.0121)	-0.0402 (-0.103,0.0227)	**0.0752 (0.00272, 0.148)**	-0.0271 (-0.0597, 0.00549)
Variable Std. Dev.	**0.476**	0.606	**NA**	**0.211**	6.18	10.0	**4.29**	3.94

Bootstrapped (BS) R-squared=0.354

Simplest Contributions Spatial Predictor Model



"Best" Contributions Spatial Predictor Model



Discussion

Key microbial sewer fate and transport factors

- Dilution to flow
- Temperature
  - Impact on amplification and attenuation
  - Seasonal effects
- Where are people contributing to the "pooled" sample

Residential (census) versus commercial (parcel) contributions

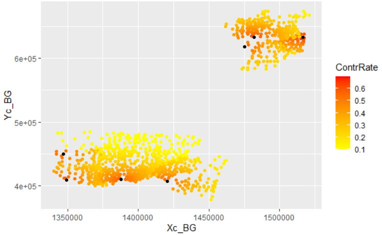
- Residential more important than commercial
  - Information about population distributions in commercially zoned areas is needed
- Temperature, but not mobility

Traits of the sewer network (e.g., combined sewer, sewer age, inflow and infiltration, industrial inputs)
Climate plays a role

Implications

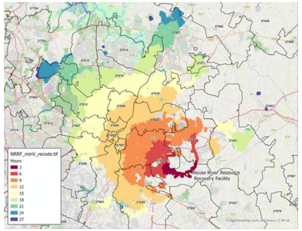
1. Wastewater measurements may not…

- represent different populations within sewersheds equally
- represent the same levels of disease prevalence during different seasons and mobility patterns
  - Monitoring long term trends given climate change?



2. Key sewershed information needed that is not publicly available:

- Extent of combined versus separate sewers
  - Inflow/infiltration information?
- At least a few estimated travel times to relate distances to travel times
- Estimated industrial flow to WWTP



3. Non-homogeneous spatial distribution of fecal concentrations across sewersheds and within sewer networks

- Decentralized wastewater treatment
- Microbial pollution potential (e.g., sewer overflow events, leaks)

https://www.neorsd.org/heavy-storms-through-cleveland-cause-overflow-prompt-swimming-advisory-at-edgewater-beach/

References

Ballesté, E.; Pascual-Benito, M.; Martín-Díaz, J.; Blanch, A. R.; Lucena, F.; Muniesa, M.; Jofre, J.; García-Aljaro, C. Dynamics of crAssphage as a human source tracking marker in potentially faecally polluted environments. *Water Res.* **2019**, *155*, 233–244.

Ahmed, W.; Zhang, Q.; Kozak, S.; Beale, D.; Gyawali, P.; Sadowsky, M. J.; Simpson, S. Comparative decay of sewage-associated marker genes in beach water and sediment in a subtropical region. *Water Res.* **2019**, *149*, 511–521.

Pruden, A.; Arabi, M.; Storteboom, H. N. Correlation between upstream human activities and riverine antibiotic resistance genes. *Environ. Sci. Technol.* **2012**, *46*, 11541–11549.

Bertels, X.; Demeyer, P.; Van den Bogaert, S.; Boogaerts, T.; van Nuijs, A. L. N.; Delputte, P.; Lahousse, L. Factors influencing SARS-CoV-2 RNA concentrations in wastewater up to the sampling stage: A systematic review. *Sci. Total Environ.* **2022**, 153290.

Costa, C. M. da S. B.; Leite, I. R.; Almeida, A. K.; de Almeida, I. K. Choosing an appropriate water quality model-a review. *Environ. Monit. Assess.* **2021**, *193*, 38.

Messier, K. P.; Akita, Y.; Serre, M. L. Integrating address geocoding, land use regression, and spatiotemporal geostatistical estimation for groundwater tetrachloroethylene. *Environ. Sci. Technol.* **2012**, *46*, 2772–2780.

Wiesner-Friedman, C.; Beattie, R. E.; Stewart, J. R.; Hristova, K. R.; Serre, M. L. Microbial find, inform, and test model for identifying spatially distributed contamination sources: framework foundation and demonstration of ruminant bacteroides abundance in river sediments. *Environ. Sci. Technol.* **2021**, *55*, 10451–10461.

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Questions?